NREL
National Renewable Energy Laboratory

Cellulose Hydrolysis and Integrated Processing Research

May 1-2, 2003

Kiran Kadam
National Renewable Energy Laboratory

Operated for the U.S. Department of Energy by Midwest Research Institute • Battelle • Bechtel

Outline

- Goals and Objectives
- Knowledge Gaps & Challenges
- Recent Experimental Work
- Recommendations for Future Work

Goals and Objectives

- Demonstrate integrated enzyme-based cellulose hydrolysis using corn stover (CS) as a model feedstock
  - Develop and apply tools to generate high-quality performance data
  - Characterize process interactions
  - Identify and bridge knowledge gaps

Major Steps in an Enzymatic Process

Lignocellulose Feedstock Collection and Delivery

↓

Pre-processing

↓

Pretreatment

↓

Conditioning →

Enzymatic Cellulose Saccharification | **Biomass Sugar Fermentation**

→ **Beer Slurry to Product and Solids Recovery**

Many options exist for each of these steps….
….and there are many interactions to consider

Knowledge Gaps & Challenges

- Knowledge gaps
  - Performance data under process-relevant conditions
    - Individual unit operations and integrated system
  - Interactions among unit operations
- Challenges
  - Achieving high yields under realistic conditions
  - Obtaining good overall and component mass balance closures

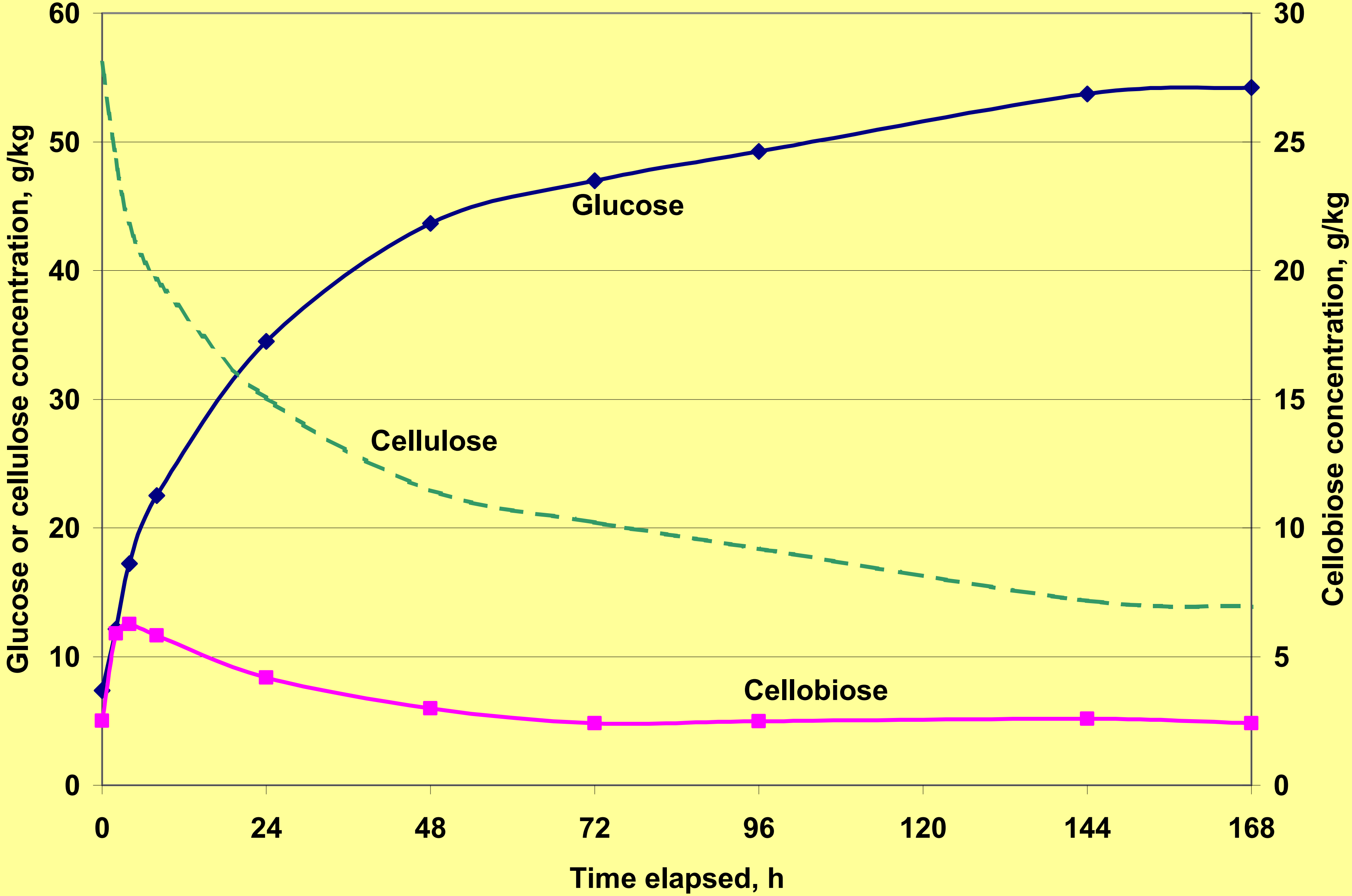
Recent Experimental Work

- Process-relevant lignocellulose saccharification
  - Reduces technical and economic risks
- Enzyme adsorption and hydrolytic performance
  - Improves understanding of cellulose hydrolysis
- Kinetic model for cellulose hydrolysis
  - Facilitates more efficient process development

Experimental System

- Pretreated corn stover (PCS): vertical Sunds reactor
- Shake flasks & bench-scale reactors
- 10%-15% (dry wt) PCS solids
- Enzyme: CPN or Spezyme
- Enzyme loading: 20-45 mg protein/g cellulose
- Temperature: 45°-55°C
- Residence time: 5-7 days

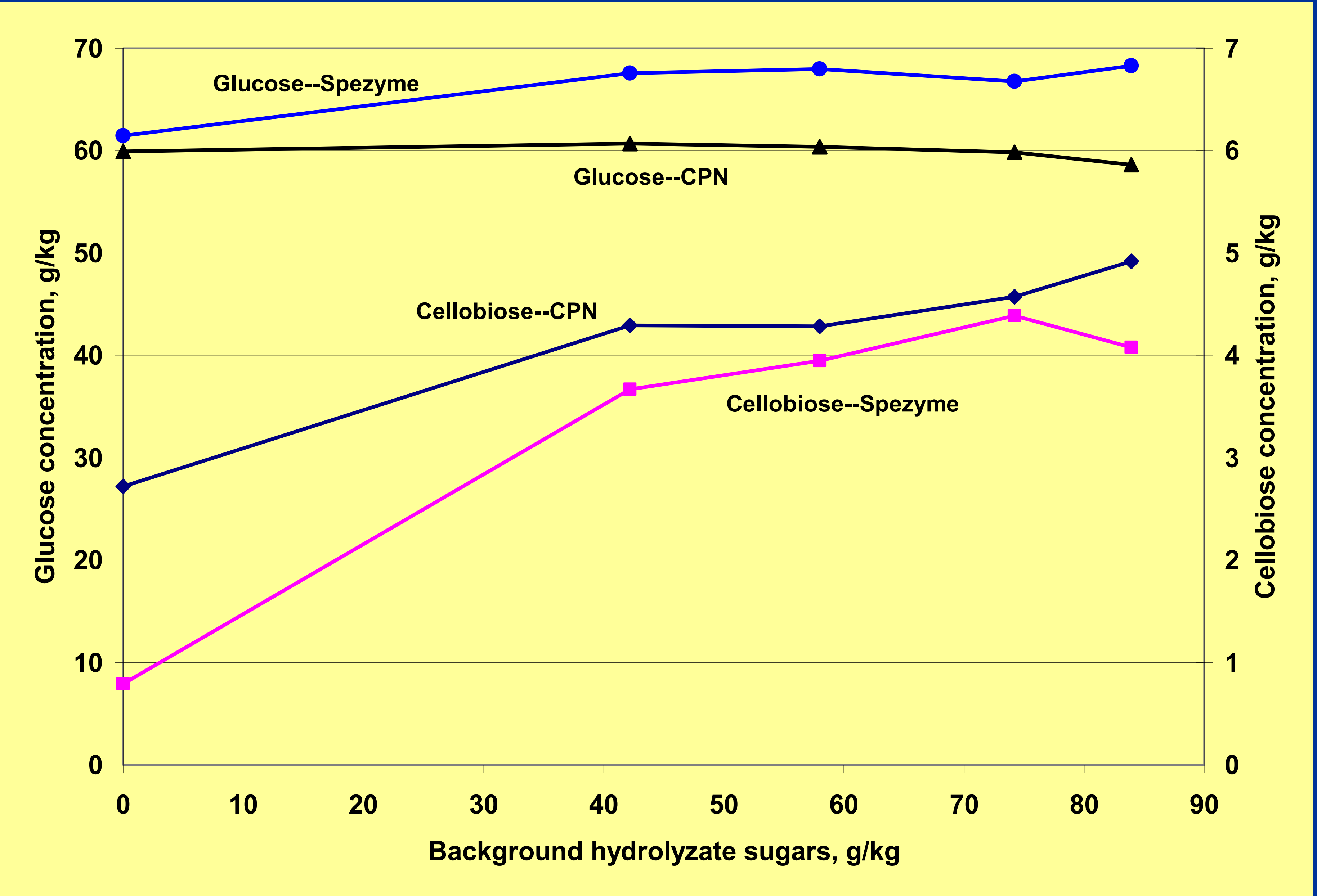
Typical Saccharification Profile with Washed PCS: 45 mg/g protein, 45°C, 10% insoluble solids



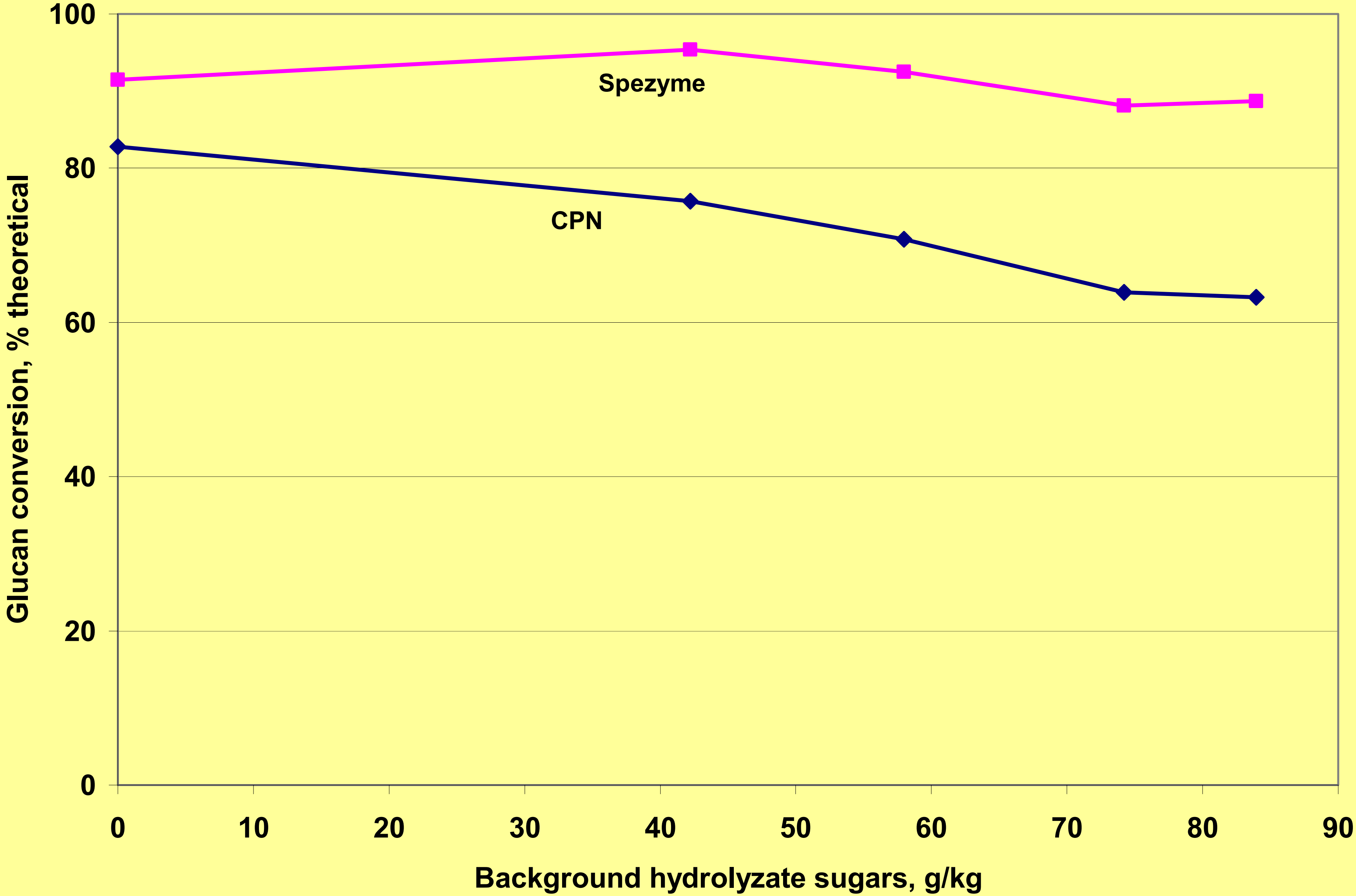
Process-Relevant Saccharification

- Extended previous work with washed PCS
- Effect of hydrolyzate conditioning on hydrolysis
  - Similar saccharification performance with neutralized or overlimed hydrolyzate
  - Neutralization used for subsequent work
- Effect of solids levels on hydrolysis
  - Shake flask system mass transfer limited at high insoluble solids levels, e.g., 15% w/w

Effect of Hydrolyzate Level: Unwashed PCS, 45 mg/g protein, 45°C, 10% insoluble solids, 7 days



Effect of Hydrolyzate Level: Unwashed PCS, 45 mg/g protein, 45°C, 10% insoluble solids, 7 days



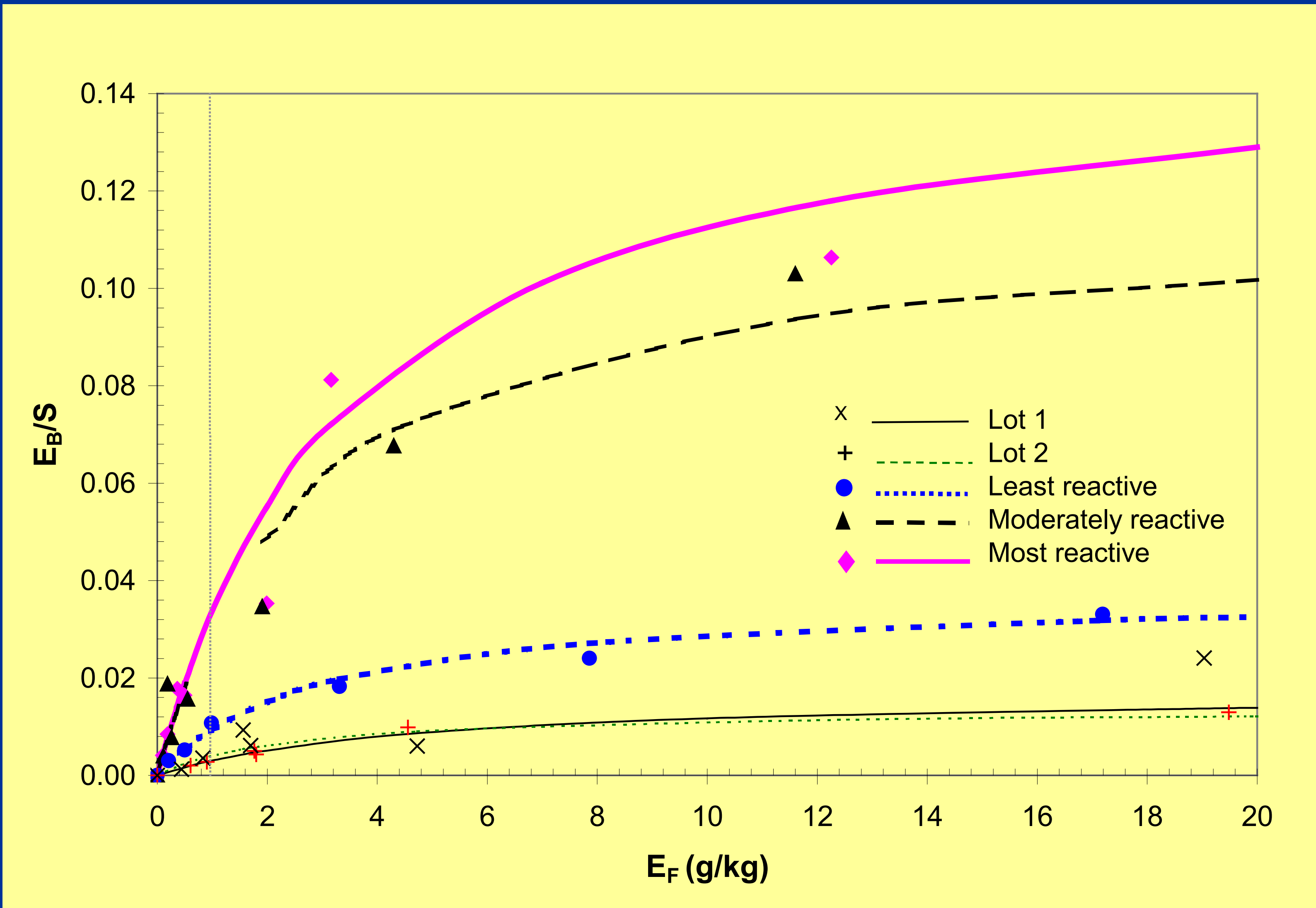
Enzyme Adsorption and Hydrolytic Performance

- Enzyme adsorption, a key factor
  - Pretreatment
  - Surface area
  - Lignin content
  - Enzyme
- Hydrolytic performance $\propto E_{max}$

$$E_B = \frac{E_{\max} K_{ad} E_F S}{K_{ad} + E_F}$$

Substrate	Glucan content, % (dry wt)
CS, Lot 1	36.9
CS, Lot 2	38.9
PCS, Lot 2 (Most reactive)	59.2
PCS, Lot 1 (Moderately reactive)	52.8
PCS, Lot 1 (Least reactive)	57.9

$$E_B = \frac{E_{max} K_{ad} E_F S}{K_{ad} + E_F}$$



Kinetic Modeling: Motivation

- Cellulose hydrolysis: major cost in the process
- Kinetic model codifies knowledge and allows *in silico* predictions
- Actual experimentation resource intensive

Baseline Kinetic Model: Key Features

- Distinguishes between the β-glucosidase and CBH/EG enzymes
- Incorporates potential inhibition by xylose
- Has structure, e.g., to potentially capture effects of
  - β-glucosidase levels
  - Temperature
  - Enzyme adsorption

Hydrolysis Reactions Modeled

Cellulose to cellobiose rx

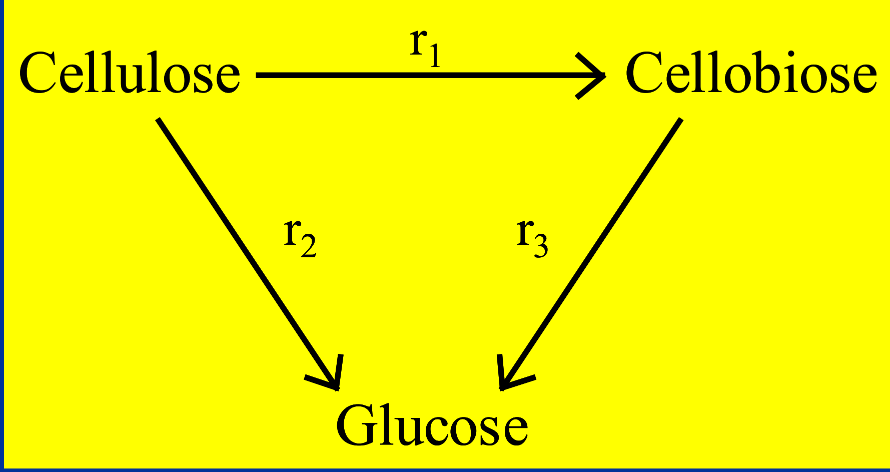
$$r_1 = \frac{k_{1r} E_{1B} R_S S}{1 + \frac{G_2}{K_{1IG_2}} + \frac{G}{K_{1IG}} + \frac{X}{K_{1IX}}}$$

Cellulose to glucose rx

$$r_2 = \frac{k_{2r} \left(E_{1B} + E_{2B}\right) S}{1 + \frac{G_2}{K_{2IG_2}} + \frac{G}{K_{2IG}} + \frac{X}{K_{2IX}}}$$

Cellobiose to glucose rx

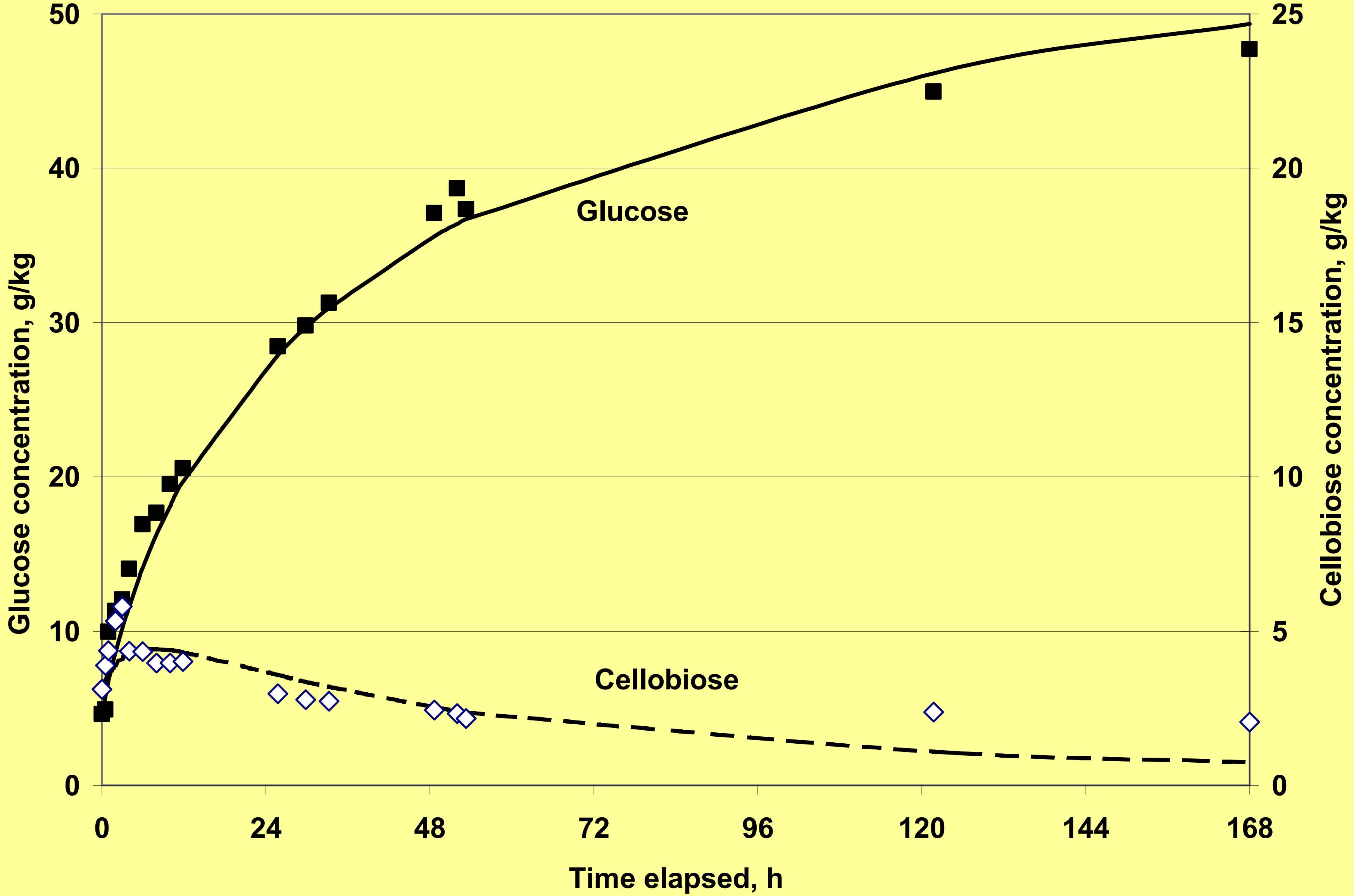
$$r_3 = \frac{k_{r3} E_{2F} G_2}{K_m (1 + \frac{G}{K_{3G}} + \frac{X}{K_{3X}}) + G_2}$$



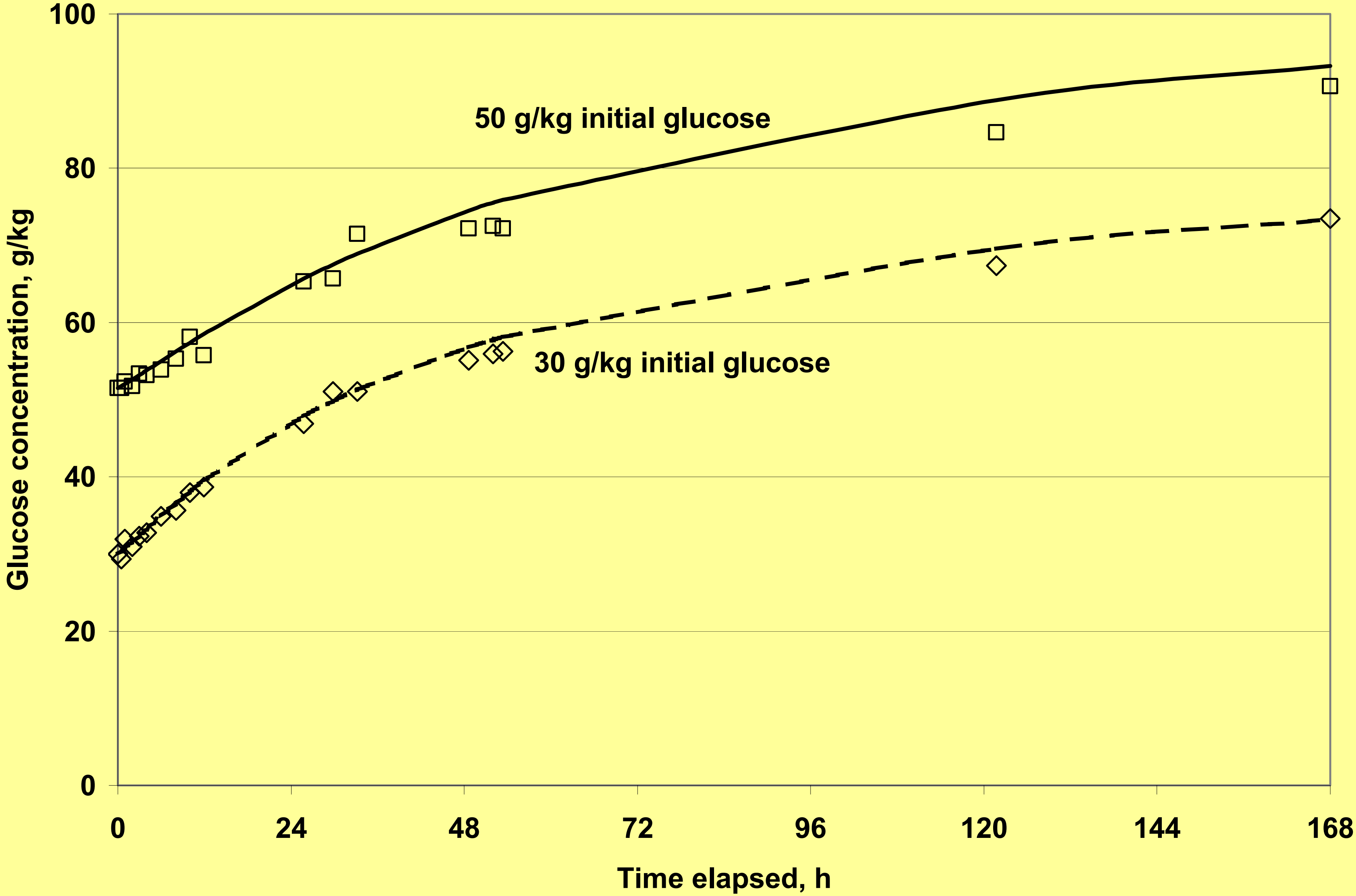
Estimated Model Parameters

Parameter	Value
Independently Established Parameters	
$K_{ad\text{-}EG/CBH}$ (g protein/g substrate)	0.4
$K_{ad\text{-}\beta\text{-}glucosidase}$ (g protein/g substrate)	0.1
$E_{max\text{-}EG/CBH}$ (g protein/g substrate)	0.06
$E_{max\text{-}\beta\text{-}glucosidase}$ (g protein/g substrate)	0.01
E_a (cal/mole)	-5540
R_s	$\alpha S/S_0$, $\alpha=1$
Parameters Obtained by Regression of Saccharification Data	
k_{1r} (g/mg hr)	22.3
K_{1IG2} (g/kg)	0.015
K_{1IG} (g/kg)	0.1
K_{1IX} (g/kg)	0.1
k_{2r} (g/mg hr)	7.18
K_{2IG2} (g/kg)	132.0
K_{2IG} (g/kg)	0.04
K_{2IX} (g/kg)	0.2
k_{3r} (hr^{-1})	285.5
K_{3M} (g/kg)	24.3
K_{3IG} (g/kg)	3.9
K_{3IX} (g/kg)	201.0

Model Validation: Washed PCS, 45 mg/g protein, 45°C, 10% insoluble solids, 40 g/kg xylose



Model Validation: Washed PCS, 45 mg/g protein, 45°C, 10% insoluble solids, 30 or 50 g/kg initial glucose



Progress in Bridging Knowledge Gaps

- Kinetic model: a new predictive tool
- Process-relevant saccharification
  - Saccharification works with neutralized hydrolyzate
  - Resistance to cellobiose inhibition a desirable trait for next generation of cellulases
- Improves understanding of configuring the overall process to maximize intermediate sugars production

Future Work: Cellulose Hydrolysis

- Evaluate 2^{nd} generation enzyme preparations under realistic conditions
  - Assess wrt conversion yields/rate assumed in process engineering model
- Evaluate other issues
  - Recommend reactor designs for effectively mixing PCS slurries
- Kinetic model
  - Extend model to include 2^{nd} generation enzymes
  - Incorporate enzyme inactivation and hydrolysis capacity factor in kinetic model
  - Use model for *in silico* process optimization

Future Work: Integrated Processing

- Characterize hydrolyzate conditioning
  - Ca, S balance
- Generate engineering data for separation processes
  - Hydrolyzate and fermentation residue
- Improve carbon/mass balance closure for individual unit operations
  - Apply new analytical tools

Future Work: Integrated Processing (Long Term)

- Integration using a model system
  - Pretreatment
  - Conditioning
  - Saccharification
  - Fermentation
- Demonstrate "robustness" under industrially relevant conditions
  - Necessary to build database for process verification
  - Reduces performance risk

Acknowledgements

- Ali Mohagheghi
- Jeffrey Knutsen
- Eric Rydholm
- Rustin Shenkman